1996 A. (1) APPLANT IN

#### SEQUENCE LISTING

## ENERAL INFORMATION:

- (i) APPLICANT: Chandrasegaran, Srinivasan
- (ii) TITLE OF INVENTION: A GENERAL METHOD TO CLONE HYBRID RESTRICTION ENDONUCLEASES USING lig GENE
- (iii) NUMBER OF SEQUENCES: 35
  - (iv) CORRESPONDENCE ADDRESS:
    - (A) ADDRESSEE: Cushman Darby & Cushman L.L.P.
    - (B) STREET: 1100 New York Avenue, NW, Ninth Floor, East Tower
    - (C) CITY: Washington
    - (D) STATE: DC
    - (E) COUNTRY: USA
    - (F) ZIP: 20005-3918
    - (V) COMPUTER READABLE FORM:
      - (A) MEDIUM TYPE: Floppy disk
      - (B) COMPUTER: IBM PC compatible
      - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
      - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
  - (vi) CURRENT APPLICATION DATA:
    - (A) APPLICATION NUMBER: US
    - (B) FILING DATE: 20-DEC-1995
    - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Kokulis, Paul N.
  - (B) REGISTRATION NUMBER: 16,773
  - (C) REFERENCE/DOCKET NUMBER: PNK/4130/213779/DJP
  - (ix) TELECOMMUNICATION INFORMATION:
    - (A) TELEPHONE: 202-861-3000
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    - (C) TELEX: 6714627 CUSH
- (2) INFORMATION FOR SEQ ID NO:1:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

54 53

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Tyr Xaa Cys Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Leu 10 15 1 5 Xaa Xaa His Xaa Xaa Xaa His 20 (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: Tyr Xaa Cys Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Leu 5 10 15 1 Xaa Xaa His Xaa Xaa Xaa His 20 (2) INFORMATION FOR SEQ ID NO:3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: Tyr Xaa Cys Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Leu 5 10 15 1 Xaa Xaa His Xaa Xaa Xaa Xaa His (2) INFORMATION FOR SEQ ID NO:4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear

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	,	'		,												
		,	•	•												
•																
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:														
	<b>V</b>	Tyr	Xaa	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa
	Xaa	1				5					10					15
•		Leu	Xaa	Xaa	His 20	Xaa	Xaa	Xaa	His							
	(2)	INFO	INFORMATION FOR SEQ ID NO:5:													
AM	(	(i)	SEQU (A) (B) (D)	LEN TYF	IGTH: PE: a	25 mino		no ac id								
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:														
$\mathcal{O}^{\circ}$		Tyr	Xaa	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa
	Xaa	1				5					10					15
		Leu	Xaa	Xaa	His 20	Xaa	Xaa	Xaa	Xaa	His 25						
	(2)	INFORMATION FOR SEQ ID NO:6:														
	` ,	(i)	(B)	JENCE LEN TYP TOP	IGTH: PE: a	26 mino	amir aci	no ac id								
		(xi)	SEQU	JENCE	DES	CRIE	PTION	J: SI	EQ II	ONO:	:6:					
		Tyr	Xaa	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa
	Xaa	1				5					10					15
		Leu	Xaa	Xaa	His 20	Xaa	Xaa	Xaa	Xaa	Xaa 25	His					
	(2)	INFORMATION FOR SEQ ID NO:7:														
		(i)	(B)	JENCE LEN TYP TOP	GTH:	25 mino	amir aci	no ac id								

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	(xi)	SEQ	JENCI	E DES	SCRII	PTIO	N: SI	EQ II	ои с	:7:					
Vaa	Tyr	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa
Xaa	1				5					10					15
	Vaa	T ou	Xaa	Vaa	ui c	Vaa	Vaa	Vaa	ui c						
	лаа	Leu	лаа	20	птэ	лаа	лаа	лаа	25						
(2)	INFO	RMAT	ION 1	FOR S	SEQ I	ID NO	3:8:								
(	(i)	SEQUENCE CHARACTERISTICS:  (A) LENGTH: 26 amino acids  (B) TYPE: amino acid  (D) TOPOLOGY: linear													
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:														
											Vaa	Dho	Vaa	Vaa	Vaa
Xaa	_	Лаа	cys	лаа		лаа	лаа	Cys	лаа		лаа	Pile	лаа	Xaa	
	1				5					10					15
	Xaa	Leu	Xaa	Xaa 20	His	Xaa	Xaa	Xaa	Xaa 25	His					
(2)	INFO	RMAT	ON I	FOR S	SEQ I	ED NO	0:9:								
	(i)	(A)	JENCI LEN TYI	NGTH:	: 27 amino	amir	no ad id								
	(xi)	SEQU	JENCI	E DES	SCRII	OITS	N: SI	EQ II	ONO:	9:					
V	Tyr	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa
Xaa	1				5					10					15
	Xaa	Leu	Xaa	Xaa 20	His	Xaa	Xaa	Xaa	Xaa 25	Xaa	His				
(2)	INFO	RMAT	ON I	FOR S	SEQ 1	D NO	0:10:	:							
	(i)		JENCI LEN												

NGTH: 23 amino aci

						o ac: linea									
	(xi)	SEQU	JENCI	E DES	SCRI	PTIOI	N: S1	EQ II	ои с	:10:					
•	Phe	Xaa	Cys	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa	Xaa
Leu	1				5					10					15
	Xaa	Xaa	His	Xaa 20	Xaa	Xaa	His								
(2)	INFO	RMATI	ON I	FOR S	SEQ :	ID NO	0:11:	:							
(	(i)		LEI TYI	NGTH:	: 24 amino		no ao id								
	(xi)	SEQU	JENC1	E DES	SCRII	PTIO	N: SI	EQ II	о ио:	:11:					
	Phe	Xaa	Cys	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa	Xaa
Leu	1		_		5					10					15
	Xaa	Xaa	His	Xaa 20	Xaa	Xaa	Xaa	His							
(2)	INFO	R <b>MAT</b> ]	ON 1	FOR S	SEQ :	ID NO	0:12	:							
			LEI TYI	NGTH:	: 25 amino		no ao id								
	(xi)	SEQU	JENCI	E DES	SCRII	PTIO	N: SI	EQ II	ои с	:12:					
	Phe	Xaa	Cys	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa	Xaa
Leu	1				5					10					15
	Xaa	Xaa	His	Xaa 20	Xaa	Xaa	Xaa	Xaa	His 25						
(2)	INFO	RMAT]	ON 1	FOR S	SEQ :	ID NO	0:13	:							

	(i)	SEQUENCE CHARACTERISTICS:  (A) LENGTH: 24 amino acids  (B) TYPE: amino acid  (D) TOPOLOGY: linear													
	(xi)	SEQU	UENC	E DE:	SCRI	PTIO	N: S	EQ II	D NO	:13:					
	Phe	Xaa	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa
Xaa	1				5					10					15
	Leu	Xaa	Xaa	His 20	Xaa	Xaa	Xaa	His							
(2)	INFORMATION FOR SEQ ID NO:14:														
(	(i)	(A) (B)	EQUENCE CHARACTERISTICS:  (A) LENGTH: 25 amino acids  (B) TYPE: amino acid  (D) TOPOLOGY: linear												
	(xi)														
Xaa		Xaa	Cys	Xaa		Xaa	Cys	Xaa	Xaa		Phe	Xaa	Xaa	Xaa	
	1				5					10					15
	Leu	Xaa	Xaa	His 20	Xaa	Xaa	Xaa	Xaa	His 25						
(2)	INFO	RMATI	ION 1	FOR S	SEQ :	ID N	0:15	:							
	(i)	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 26 amino acids  (B) TYPE: amino acid  (D) TOPOLOGY: linear													
	(xi)	SEQU	JENCI	E DES	SCRII	PTIO	M: S]	EQ II	ON C	:15:	,				
Vaa	Phe	Xaa	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa
Xaa	1				5					10					15
	Leu	Xaa	Xaa	His 20	Xaa	Xaa	Xaa	Xaa	Xaa 25	His					
					,										

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	(i)	(A)	JENCI LEN TYI	NGTH:	25	amiı ac:	no ao id								
	(xi)	SEQU	JENCI	E DES	SCRII	PTIO	N: S1	EQ II	ONO:	:16:					
Vaa	Phe	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa
Xaa	1				5					10					15
	Xaa	Leu	Xaa	Xaa 20	His	Xaa	Xaa	Xaa	His 25						
(2)	INFORMATION FOR SEQ ID NO:17:														
	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 26 amino acids</li><li>(B) TYPE: amino acid</li><li>(D) TOPOLOGY: linear</li></ul>														
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:														
W	Phe	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa
Xaa	1				5					10					15
	Xaa	Leu	Xaa	Xaa 20	His	Xaa	Xaa	Xaa	Xaa 25	His					
(2)	INFO	RMATI	ON I	FOR S	SEQ ]	ED NO	):18	:							
	(i)	(A)	JENCI LEN TYI TOI	NGTH:	27	amii o ac:	no ao id								
	(xi)	SEQU	JENCI	E DES	SCRII	PTIO	N: S	EQ II	ON C	:18:					
	Phe	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa
Xaa	1				5					10					15

(2) INFORMATION FOR SEQ ID NO:16:

W 5%

Xaa Leu Xaa Xaa His Xaa Xaa Xaa Xaa His 20 25

- (2) INFORMATION FOR SEQ ID NO:19:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 39 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

NNNNNNNNN NNNNNNNNN NNNNNGAGGA GGCTNNNNN 39

- (2) INFORMATION FOR SEQ ID NO:20:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 39 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

NNNNNNNNN NNNNHNNNN NNNNNGAGGG ATGTNNNNN 39

- (2) INFORMATION FOR SEQ ID NO:21:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 74 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAGCTCCTTT GGATCCAAGC TTCCCGGGGA AGAATTCGAG GAGGCTCTCG AGGTCGACTT 60

CCTCTAGAGG TACC

74

- (2) INFORMATION FOR SEQ ID NO:22:
  - (i) SEQUENCE CHARACTERISTICS:

(1) 60

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- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
- GAGCTCCTTT GGATCCAAGC TTCCCGGGGA AGAATTCGAG GAGGCTCTCG AGGTCGACTT
  60

CCTCTAGAGG TACC 74

- (2) INFORMATION FOR SEQ ID NO:23:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 74 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
- CUCGACCAAA CCUAGGUUCG AAGGGCCCCU UCUUAAGCUC CUCCGAGAGC UCCAGCUGAA

GGAGAUCUCC AUGG 74

- (2) INFORMATION FOR SEQ ID NO:24:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 87 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
- GGATCCAAGC TTAGCGATCT GCCTGCAGGT CGACTCTAGC CAGGGGCGGG GTGGTCTAGT 60
- ATCGTTCAAT GATACTTCAT GGAATTC
  87
- (2) INFORMATION FOR SEQ ID NO:25:
  - (i) SEQUENCE CHARACTERISTICS:

6221

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- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCCCTGAAGG AGATATACAT ATG 23

- (2) INFORMATION FOR SEQ ID NO:26:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 25 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGACTAGTCC CTTCTTATTC TGGTG 25

- (2) INFORMATION FOR SEQ ID NO:27:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 15 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser

- (2) INFORMATION FOR SEQ ID NO:28:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 14 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

362

### CTGACGGGGG CCAA

14

- (2) INFORMATION FOR SEQ ID NO:29:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 15 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

#### CTAGTTGGCC CCCGT

15

- (2) INFORMATION FOR SEQ ID NO:30:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 24 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

#### CTAGACGGGG GAGGCGGCAG TCAA

24

- (2) INFORMATION FOR SEQ ID NO:31:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 24 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

#### CTAGTTGACT GCCGCCTCCC CCGT

24

- (2) INFORMATION FOR SEQ ID NO:32:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 5 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Gly Gly Ser 1 5

- (2) INFORMATION FOR SEQ ID NO:33:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 39 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTAGACGGGG GAGGCGGCAG TGGAGGTGGC GGATCACAA

- (2) INFORMATION FOR SEQ ID NO:34:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 39 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
  CTAGTTGTGA TCCGCCACCT CCACCGTCGC CTCCCCCGT
  39
- (2) INFORMATION FOR SEQ ID NO:35:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 10 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Gly Gly Ser Gly Gly Gly Ser 1 5 10

(1)

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# NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUMBER OF SEQUENCE NO/OR AMINO ACID SEQUENCES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821 - 1.825 for the following reason(s): 1. This application clearly fails to comply with the requirements of 37 CFR 1.821 - 1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990. 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c). 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821( 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached marked-up copy of the "Raw Sequence Listing." 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d). 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 CFR 1.821(e). 7. Other: Applicant must provide: An initial or substitute computer readable form (CRF) copy of the "Sequence Listing" An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification A statement that the content of the paper and computer readable copies are the same and, where applicable, include n new matter, as required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d) For questions regarding compliance with these requirements, please contact: For Rules Interpretation, call (703) 308-1123 For CRF submission help, call (703) 308-4212

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